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accuracy, completeness and utility of the illustrations to the clinician and practitioner, the broad biological conception underlying the treatment, combine to characterize the work as the best iconography of parasitology as yet published.

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THE RELATION BETWEEN LIZARDS AND
PHLEBOTOMUS VERRUCARUM AS
INDICATING THE RESERVOIR
OF *VERRUGA*

It affords the writer much satisfaction to record another confirmation of the intimate relation which exists between *Phlebotomus* and lizards or other reptiles the world over. Many cases of this relation have been recorded in the recent literature, and the same appears to hold good in Peru.

Numerous blood smears made during the past two or three months from small rock lizards of several species collected at Verrugas Canyon, Surco, San Bartholomé and Chosica Canyon all show rod and granule bodies which exhibit the identical morphology of the bodies that have been named *Bartonella bacilliformis*. Their agreement with the latter in shapes, sizes, colors and apparent structure is so faithful as to defy distinction. The lizards concerned have been sent in for identification.

It is to be noted that the first three localities above mentioned are well within the limits of the verruga zone of the Rimac Valley, while Chosica Canyon is just outside that zone. Lizard blood smears made in Chosica Canyon in June, 1913, and again recently all show these bodies, but the granules seem to predominate greatly in the blood of the lizards from outside the verruga zone and from points within the zone where the lizards are not exposed to the constant attacks of the *Phlebotomus*.

In Verrugas Canyon there are, close to the house, many large walls built of loose rock wherein the *Phlebotomus* hide in swarms during the day, issuing in the evening to enter the house and bite the inmates. These rock walls are also inhabited by the small lizards in ques-

tion. Smears of blood made from lizards from these walls show a great predominance of the rods over the granules. These lizards are exposed to the constant attacks of the *Phlebotomus* every day in the year.

The writer has found the same bodies in smears made from the *Phlebotomus* at Verrugas Canyon, which also show the nucleated red corpuscles of the lizards as well as mammalian erythrocytes. The same rods and granules have furthermore been found by the writer in microtome sections of human verruga papules, in similar sections of papules produced in his laboratory animals by injections of the *Phlebotomus*, and in the blood of these animals prior to the eruption.

Blood smears of a young guinea-pig taken 63½ hours, and later, after injection subcutaneously with a very small quantity of citrated lizard blood from Chosica Canyon have shown the typical granules and Bartonella rods in the disks of the erythrocytes. This pig died nine days after injection, after irregular rises of temperature, and its autopsy blood and femoral marrow showed a large increase of the bodies, principally granules but also short rods.

Subcutaneous injection of a second young guinea-pig with a larger quantity of citrated lizard blood from Surco proved fatal within ten hours, liver smears showing the rods and granules, but blood, marrow and spleen smears proving practically negative. Further experiments of a similar nature are under way. The three-cornered connection, however, between lizards, *Phlebotomus* and verruga appears to be already well established by these data.

It is seen from the results that this possible reservoir of verruga in the lizards is not confined to the verruga zones, which are limited by the occurrence of the *Phlebotomus*, but may exceed the latter in range. This explains how fluctuations in occurrence of the *Phlebotomus* may result in extensions or retractions of the verruga zones, the gnats finding the infection at hand on gaining a new locality.

It also seems indicated by the above results that the verruga organism must exist in the infective stage in the lizard blood and does not apparently demand the medium of the *Phlebo-*

tomus for its development but only for its transfer to new hosts. Thus the *Phlebotomus* appears to be merely a mechanical transmitter of verruga, and not a true secondary host of the organism. But it is probable that the gut of the *Phlebotomus* favors the free liberation of the infective stage of the organism, which either penetrates thence to the salivary glands or passes directly forward through the alimentary canal, by regurgitation or otherwise, to the pharynx and thus gains the proboscis.

It seems demonstrated by the above findings that these small rock lizards constitute at least one reservoir of verruga. Whether snakes, man or other mammals constitute additional reservoirs of the disease remains to be determined. The writer puts forth the tentative opinion, subject of course to future modification, that lizards and possibly snakes, in other words reptilian animals of cold blood, may yet be found to constitute the sole reservoir of verruga. It seems quite possible that *Phlebotomus* can not become infected with verruga from the blood of mammals, but this point needs careful investigation.

As bearing on this view, it is to be noted that no one has yet succeeded in making cultures of the *Barton* bodies from human blood, and that injections of mammalian blood containing these bodies have given only negative results thus far. The verruga organism might be looked upon as outside its natural environment in mammalian blood, but at home in reptilian hosts. Nevertheless it is quite possible that it has been overlooked in the experiments after making the injections of *Barton* containing blood referred to.

The above rods and granules have also been found by the writer in the bone marrow, liver and spinal cord of the lizards, as shown by smears from these organs. In the blood of the lizards the rods and granules are often free in the plasma but frequently in or attached to the surface of the red corpuscles. They stain with Giemsa characteristically brownish, sometimes bluish or reddish, exactly as do the *Barton*. If these organisms are not identical with the *Barton*, they are certainly very similar morphologically and evidently bear a con-

stant relation to verruga. It has not been possible as yet to attempt cultures of these bodies with the view of demonstrating their nature, owing to lack of both time and facilities. They may easily turn out to be the bacillus paratyphoid B, in large part at least, but in any case they seem linked with *Barton* in some thus-far mysterious relation.

There is quite a large possibility that the *Barton* may prove to be simply the lizard-blood bodies parasitized by the verruga organism proper. From 1900 to 1902 Barton demonstrated the bacillus paratyphoid B in all his verruga cases, and with it he produced the fever and eruption in both dogs and mules. Since this bacillus is so constantly present in verruga cases it seems certain to the writer that it bears some important relation to the disease. As it has been cultivated with ease, while *Barton* has not, it may well be the case that the latter is simply an infected form of it which has lost its reproductive power. In such event the verruga organism does not reach the infective stage until the *Barton* containing it has broken up naturally and disappeared. Barton's animal experiments seem strongly to indicate that the bacillus paratyphoid B carries verruga infection.

Similar cases of the constant attendance of certain bacilli upon diseases of obscure etiology, as yellow-fever, hog-cholera, etc., are well known. It may well be that such bacilli are infected with the respective ultramicroscopic organisms of these diseases and play an important rôle in their carriage.

Whether the intracorpuseular bodies found by Laveran and Carini in the blood of lizards are of the same type as the present rods and granules remains to be seen. It would seem quite likely that the two may be closely related. The present bodies, which are only tentatively assumed to be *Barton* or to metamorphose abnormally into the latter, exhibit much resemblance to *Theileria*. The granule stage also approaches the marginal-point stage of *Anaplasma*, and is very similar to the stages figured by Anderson for the Rocky Mountain spotted-fever organism, which is probably not a *Piroplasma*.

Blood smears of a native rat, probably a species of *Euneomys*, caught at Verrugas Canyon, have shown nothing definite. Smears of the blood of dogs and burros, doves and ground-owls from the same locality have likewise proved negative. The vizcachas, *Viscaccia* spp., are contraindicated as a reservoir of verruga. It has not yet been practicable to secure vizcacha blood smears from the verruga zone, but these animals do not occur close to the house in Verrugas Canyon, where the *Phlebotomus* is very abundant in the rock walls, which it evidently leaves only to enter the house or attack persons and animals close by. Therefore these particular gnats are precluded from deriving their infection from the vizcacha, and they are well known to be infected at most times if not continuously.

In conclusion it may be pointed out that, on *a priori* grounds, the inference is logical that the lizards constitute a verruga reservoir. The *Phlebotomus* passes the daylight hours within the darkened recesses of the loose stone walls and piles of rock in order to escape wind and strong light. Lizards inhabit the same places, finding their food there and coming out only briefly at rare intervals to sun themselves. The *Phlebotomus* is always ready to suck blood in the absence of light and wind, and has been found more prone to suck reptilian than mammalian blood. Nothing is more natural than that the *Phlebotomus* should suck the blood of the lizards to a large extent during the day, and this is what actually happens. If the *Phlebotomus* carries verruga, and this is already demonstrated to be the fact, it follows that the lizards must become infected therefrom even if they were not originally so. That they are probably the original reservoir of the disease is indicated in general by the constant host relation which obtains between *Phlebotomus* and reptiles the world over and specifically by the mutual habitat of the two which has resulted in their being thrown continually together since their existence began.

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CHOSICA, PERU,
April 27, 1914

SPECIAL ARTICLES

ON THE ANTAGONISTIC ACTION OF SALTS AND ANESTHETICS IN INCREASING PERMEABILITY OF FISH EGGS (PRELIMINARY NOTE)

In previous papers¹ it was shown that pure salt solutions and nicotine increased the permeability of fish eggs and that these permeable eggs developed abnormally, giving rise to cyclopia and other abnormalities common to fish embryos. During the present season I have observed a few cyclopic or one-eyed pike embryos in the hatching jars of the State Fish Hatchery, St. Paul, Minnesota. Eggs of the pike and muskalonge were found to live in water re-distilled in quartz and to be adaptable to permeability experiments. Pike eggs were used, and although they were not as impermeable as *Fundulus* eggs, they were normally but very slightly permeable to salts. They were placed in distilled water and in solutions of anesthetics or of sodium nitrate, and the chlorides diffusing out of them estimated quantitatively with the nephelometer. Except for the use of the nephelometer, which admitted of a quantitative estimation of very minute quantities of chlorides, the technique was the same as given in the previous papers. If one or more eggs died in an experiment, it was repeated. Pike eggs will live in 3 per cent. alcohol for many days and in 6 per cent. alcohol for a considerable length of time.

Six per cent. alcohol, or $\frac{1}{2}$ saturated (more than 1 per cent.) ether, or $\frac{1}{10}$ molecular sodium nitrate increased the permeability of the eggs. This change was irreversible, but did not kill the eggs—after the eggs were put back into distilled water they remained permeable.

When a salt and an anesthetic were combined in the same solution, it was found that the anesthetic antagonized the action of the salt. This antagonism was not very marked, but seemed to be constant. The method of procedure is shown by the following example: A mass of pike eggs was divided into three exactly equal lots. Lot 1 was placed in 50 c.c. $\frac{1}{10}$ molecular NaNO_3 . Lot 2 in 50 c.c. $\frac{1}{10}$ molecular NaNO_3 containing 3 per cent. alcohol.

¹ McClendon, SCIENCE, N. S., Vol. 38, p. 280; and *Internat. Zeitsch. f. Physik.-Chem. Biologie*, 1914, Vol. 1, p. 28.